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Abstract

The childhood-onset genetic motor neuron disease spinal muscular atrophy (SMA) is characterized by progressive weakness, muscle wasting and, for the most severely affected patients, premature death. SMA is caused by loss of *SMN1*, leading to low levels of full-length survival motor neuron (SMN) protein. Excitingly, the disease-modifying therapy nusinersen (marketed by Biogen as Spinraza™), has recently been approved for SMA. Nusinersen is an antisense oligonucleotide (ASO) delivered intrathecally to specifically correct the genetic defect that underlies SMA and restore SMN protein levels. Many SMA patients benefit greatly from nusinersen and other forms of SMN-targeted therapies that are currently in various stages of clinical development. However, initial clinical studies also illustrate several limitations of SMN-targeted therapies, including the requirement for a very early treatment start and uncertainty around the long-term efficacy of targeting treatment exclusively to the central nervous system. Here, I will discuss possible further avenues for SMA therapy development beyond current SMN-targeted therapies, focusing on how basic and clinical lines of research can inform and support each other to optimize future therapy development.

1. Introduction

Spinal muscular atrophy (SMA) is a motor neuron disease leading to progressive weakness, muscle wasting and, for the most severely affected patients, premature death [1]. SMA is caused by homozygous deletion of, or loss-of-function mutations in, the *survival motor neuron 1* gene (*SMN1*). A second *SMN* gene (*SMN2*) that is alternatively spliced partially compensates for this loss, but only produces low levels of full-length SMN protein [2]. Since this discovery, combined efforts from researchers, clinicians and the SMA patient community have led to the development and recent approval of a disease-modifying therapy, nusinersen (marketed by Biogen as Spinraza™). Nusinersen is an antisense oligonucleotide (ASO) delivered directly into the central nervous system (CNS) by intrathecal injection to specifically correct the genetic defect that underlies SMA by targeting *SMN2* splicing. Similarly, other SMN-targeted therapies that aim to increase SMN levels either by correcting *SMN2* splicing or replacing *SMN1* are currently in various stages of development.

Early findings from clinical trials and subsequent follow-up studies indicate that many SMA patients benefit greatly from nusinersen and other forms of SMN-targeted therapies [3-5]. However, the same studies also illustrate several limitations of SMN-targeted therapies. For example, not all patients benefit equally from SMN-targeted therapies, and early commencement of treatment provides most benefit, but is not always feasible. These findings are generally in line with previous preclinical studies, which showed that there is a limited therapeutic window available to start SMN-targeted therapy in animal models of SMA [6]. Importantly, preclinical studies also indicated that CNS-specific restauration of SMN was not sufficient to provide complete rescue of the SMA phenotype in animal models [7], which suggests that current nusinersen therapy for SMA may lead to the development of further, non-CNS symptoms in SMA patients as they get older. Therefore, the approval of nusinersen represents an important moment for the SMA research field to determine and discuss key questions that need to be addressed in the coming years in order to provide optimal therapies and support. Here, I will focus on possible avenues for SMA therapy development beyond current SMN-targeted therapies.

2. Dissecting the cellular consequences of SMN depletion

Because the genetic cause of SMA has long been known, significant research efforts have focused on understanding the cellular changes that occur when levels of SMN are reduced. Initial studies linked SMN to the biogenesis of small nuclear ribonucleoproteins (snRNPs), the

protein-RNA complexes that are required for spliceosome formation [8]. This was later extended to general RNP biogenesis, as SMN was shown to be involved in the formation of a number of similar RNP complexes [9]. Because of this role, the function of SMN in RNA splicing has been extensively studied. Although SMN plays an important role in splicing, splicing defects in SMA appear to occur late in pathogenesis and might not be directly linked to cell type specific pathology, although this remains to be definitively determined [10,11].

Since, SMN has been shown to be involved in a wide range of cellular pathways that are required for neuronal and synaptic homeostasis. These pathways include endocytosis, cytoskeletal dynamics, mitochondrial function and ubiquitin homeostasis [12-18]. Current issues in SMA research that will need to be addressed is the question of whether and how these pathways are related, to what extent SMN plays a central role in each of these pathways, and whether other master regulators or modifiers exist that interact with or control SMN function (**Figure 1**). For this to be successful, it will be important to first better understand the biological role of SMN independent of SMA, as it will allow identification and dissection of key cellular pathways in a non-disease context [19]. In trying to identify molecular mechanisms that link previously identified SMN-dependent molecular mechanisms, an interesting hypothesis is that of altered protein homeostasis as a central regulator of disease pathways in SMA. Correct protein turnover by local translation and degradation is required for neurons and synapses to function properly, and interestingly, both these pathways have been shown to be affected in SMA and to depend on SMN [15,20,21]. However, the exact role of SMN in both pathways remains to be determined in more detail and is likely to involve a complex interplay between multiple cellular pathways and mechanisms.

3. Using insights from basic research to inform further clinical development

Considering the emerging limitations of SMN-targeted therapies and the broad range of cellular pathways that have been linked to SMN function and SMA pathology, SMN-targeted therapies will likely need to be combined with other therapeutics that target other proteins and pathways to provide all-round treatment and stop disease progression. Indeed, a significant number of such proteins and pathways have already been identified in SMA (see section 2 and e.g. [22]). As the number of SMA patients is relatively small, careful consideration will be required to determine which targets should eventually be taken forward into clinical studies. This will require a pragmatic approach as certain targets may be very promising in animal models, but technically and pharmaceutically challenging to implement in human patients. This further emphasizes the need for studies that aim to identify central regulators of SMA

pathology and the precise hierarchy between pathways (**Figure 1**). Moreover, it will be necessary to develop improved animal models that better reflect the post-nusinersen clinical landscape, i.e. models that are based on genetic depletion of SMN followed by postnatal pharmacological restoration of SMN expression (for example [23]). Standardizing these models across labs and across the main mouse models for SMA is likely to be an important point of focus for the SMA field in coming years, including the optimization of more sensitive phenotypic readouts for mouse models that allow to reliably assess neuromuscular function at a range of developmental stages. These preclinical studies will have to be complemented by more detailed clinical phenotyping of SMA patients, including comparative studies of the natural history of SMA patients before and after nusinersen approval. Also, current outcome measures for clinical trials may need further refinement in order to successfully investigate combinatorial therapies. This may include further optimization of existing tests but may also include the development of further biomarkers based on lab- and imaging-readouts to expand current possibilities for more refined measurements of disease state and progression. As before, extensive crosstalk between preclinical and clinical lines of research will remain vital to successfully move the SMA field forward.

4. Conclusion

Research in recent years has led to a substantially increased understanding of SMA. The diversity of novel SMN-associated molecular mechanisms, and cellular pathways affected in SMA illustrates the complexity of the processes that underlie motor neuron degeneration. This complexity forms a challenge, but also provides more starting points for understanding SMA pathology and identify further therapeutic targets. Combined with evolving knowledge on the long-term effect of SMN-targeted therapies, the SMA field is likely to develop considerably in years to come.

5. Expert opinion

The development and approval of nusinersen represents a crucial and historic moment in SMA research, comparable to the discovery of *SMN1* deletion as the cause of SMA and the discovery of alternative splicing of *SMN2*. SMA research leading up to the approval of nusinersen has been relatively straightforward, as it focused on how to specifically target a single genetic defect and increase patient survival. Moving forward, SMA research will enter a more complex phase, in which novel therapies will need to be compared to existing ones.

Moreover, future research will need to consider the overall health and quality of life of patients, and as such look for more subtle changes, all in a relatively small and limited group of vulnerable patients.

In the past, developments in understanding the genetic basis of SMA have guided SMA research. Similarly, the consequences of the approval of nusinersen will have to guide current and future research, thereby refocusing and informing priorities. Clinically, the observation, characterization and follow-up of patients that have been treated with nusinersen and possible future SMN-targeted therapies will be of vital importance. These studies will need to lead to the development of more detailed outcome measures for therapeutic studies and better insights into patients' expectations and requirements that are likely to arise from changing disabilities. Scientifically, the SMA field will need to update existing, and develop novel, model systems that better reflect the new patient situation and improve the understanding of how cellular pathways that are affected in SMA are related. Combined, this is likely to lead to a shift of focus in therapy development from survival to symptoms and quality of life, and from SMN replacement to SMN-combination therapies. This will broaden current SMA drug development avenues and eventually lead to an extended arsenal of therapeutic options for SMA patients that goes beyond replacing SMN.

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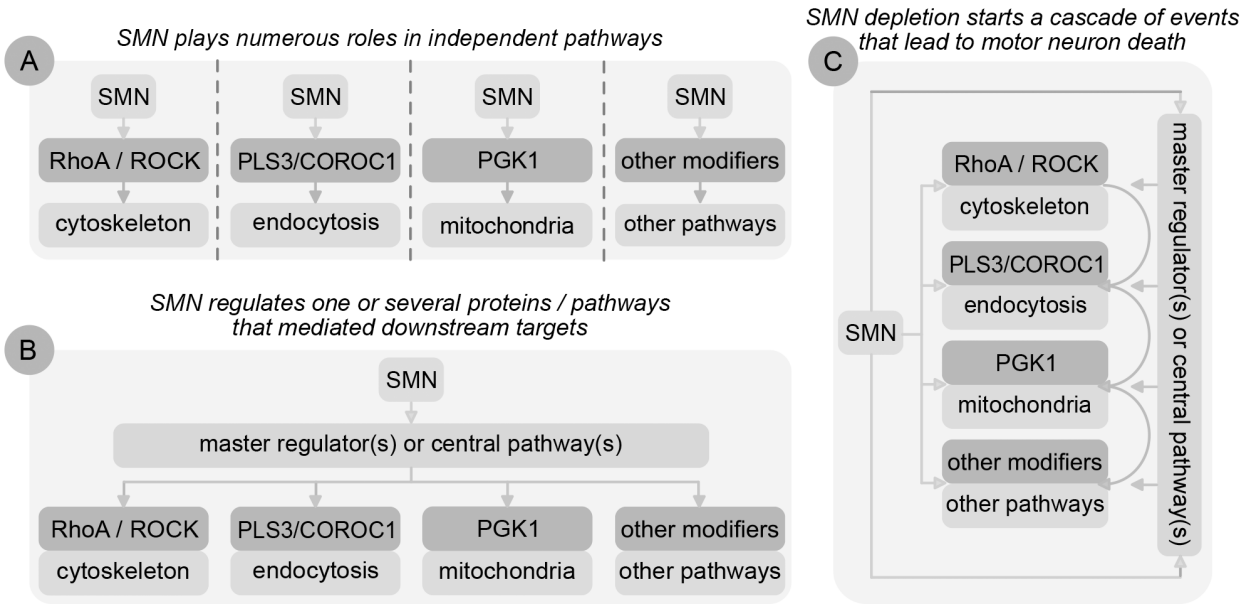

Figure 1. *Possible involvement of SMN protein in cellular pathways and downstream consequences*.

Many cellular pathways have been linked to SMN function and SMA pathogenesis. What is currently unknown, is how and if these pathways are functionally related. For this, several possibilities can be proposed, each requiring further investigation. First, SMN could separately regulate or be involved in multiple cellular pathways (**1A**). Second, SMN might regulate one or several master regulators or central pathways, such as protein translation and degradation, that in turn affect the function of multiple, downstream pathways (**1B**). Finally, SMN depletion in SMA might start a cascade of cellular defects that could be further regulated by central pathways or other proteins and form a combined cause of pathology (**1C**). Many modifiers of SMA pathogenesis have already been identified (see e.g. [22]) and therefore RhoA/ROCK, PLS3/COROC1 and PGK1 serve as examples of distinct pathways that have been shown to be affected in SMA, rather than providing an exhaustive list.

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This study provides an important example of how next-generation animal models for SMA that are based on partial SMN-restoration can be generated.